Experience with Ferrous Bis-Glycine Chelate as an Iron Fortificant in Milk

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Abstract: The objective of this study was to test whether milk is an appropriate vehicle for fortification with ferrous bis-glycine chelate and whether it has any effect on milk's organoleptic properties. In addition, the study examined the children's acceptability and tolerance of the fortifying agent. One hundred thirty-one children aged 6–14 years (79 males and 52 females) from two dormitories of the Ministry of Social Welfare in Riyadh City, Saudi Arabia participated in this study. The results of this trial showed that milk fortified with this iron chelate has unaltered organoleptic properties and is well accepted by the children. Hemoglobin and serum ferritin levels were measured before and after consuming one liter of milk fortified with 30 mg ferrous bis-glycine chelate per liter (6 mg elemental iron per liter) per day, for a period of three months. The prevalence of anemia (Hb < 12 g/dL) significantly dropped from 25.3 to 5.0%, and 23.0 to 9.6%, among boys and girls respectively. The prevalence of low serum ferritin values among boys dropped from 8.8 to 5.9% and significantly from 21.1 to 12.1% among girls. No control group was included in this study. It is concluded that ferrous bis-glycine chelate in milk does not alter milk's organoleptic properties; furthermore, it improved hemoglobin and ferritin serum levels among anemic children, suggesting milk as an appropriate vehicle for fortification with this chelate.

Key words: Fortified milk, ferrous bis-glycine chelate, iron deficiency anemia

Introduction

Iron deficiency anemia (IDA) is highly prevalent worldwide, especially in developing countries. The most critically affected are infants, school-aged children, and women of reproductive age [1–3]. In spite of several programs that have been implemented to control iron deficiency anemia in many parts of the world, its prevalence is still not declining [4, 5].

The kingdom of Saudi Arabia is not exceptional in its high prevalence of anemia. The National Nutrition Survey (NNS) among the Saudi people [6] showed that the prevalence of anemia ranged between 13 and 18.5% among the populations of different parts of the kingdom; the most affected groups were young children and females of reproductive age. Iron deficiency, parasitic infestation, and genetic predisposition were reported among the common causative factors [7–10]. Al Othaimeen *et al* [11] reported IDA (Hb < 12 g/dL) prevalence of 35–54% among schoolgirls aged 7–14 years.

Lozoff [12] reported that iron deficiency among children may cause some alterations in cognitive ability, and lower developmental intelligence quotient and achievement test scores. As a consequence of the many deleterious effects of IDA, fortification of food with iron is considered the best sustainable method of preventing iron deficiency [13].

Most well-absorbed iron compounds that are usually used for food fortification, of which ferrous sulfate is an example, have adverse organoleptic effects such as color change, oxidative rancidity, an off flavor, and precipitation in liquid and semi-liquid preparations [14]. Attempts have been made to find iron compounds that are not susceptible to chemical changes and with bioavailability that is not significantly impaired by substances that form complexes in the gastrointestinal tract, such as phytate and polyphenols [15]. One such compound that has been developed is iron glycine chelate [16].

Several trials were carried out to determine the bioavailability of iron glycine chelate. Fairweather-Trait *et al* [17] found iron from iron glycine chelate to be more readily utilized than iron from ferrous sulfate in rats. Weanling rats fed iron-fortified, casein-based formula absorbed more iron from ferric glycinate than from ferrous sulfate [18]. However Olivares *et al* [19] showed that the absorption of iron from ferrous glycine chelate-fortified milk in women was significantly lower than when the same fortificant was given in water. They concluded that milk inhibits the bioavailability of iron from bis-glycinate, which is still 2–2.5 times more available than is iron from ferrous sulfate added to milk. Stekel *et al* [20] also indicated that iron absorption from cow's milk fortified only with iron bis-glycine chelate is comparable to that obtained in milk fortified with ferrous sulfate plus ascorbic acid, and that the addition of vitamin C to iron glycine chelate improved iron bioavailability. However the enhancing effect of ascorbic acid on iron absorption of milk fortified with iron bis-glycine was proportionately lower than that obtained in milk fortified with ferrous sulfate, showing that iron-amino acid chelate is less influenced by the action of ascorbic acid [20].

Hurrell [21], however, after reviewing the published data, came to the conclusion that iron bis-glycine chelate was equivalent to ferrous sulfate bioavailability. In addition, Fox *et al* [22] reported that there was no difference between the bioavailability of iron from ferrous sulfate and that from ferrous bis-glycine added to a high-phytate cereal weaning food fed to children. More recently, Bovell-Benjamin *et al* [23] compared iron absorption from ferrous bis-glycinate and ferrous sulfate in iron-sufficient men. They showed that iron from bis-glycinate was about four times better absorbed than iron from ferrous sulfate in whole maize meal high in phytate.

The absorption of the chelate was reported to be well regulated by body iron stores [19]. Tolerance studies have shown that it is well tolerated and does not produce gastric discomfort [24]. The objective of this study was to investigate if milk is an appropriate vehicle for fortification with ferrous bis-glycine chelate and to assess its acceptability and tolerance by Saudi children.

Subjects and Methods

Selection of vehicle for fortification

In the past, the staple diet of Saudis consisted of whole wheat meal prepared dishes, milk and dairy products, and dates. To the present time, milk has been a mainstay of Saudi meals. The NNS [6] revealed that milk consumption is very frequent, and *per capita* consumption is not less than two cups daily. This makes it an appropriate vehicle for fortification. Ferrous bis-glycine chelate (Albion Laboratories Inc., Clearfield, Utah, USA) was used as fortificant. Ferrochel is the commercial name for this compound. It contains, in addition to ferrous-bis-glycinate, cabosil (silicone dioxide added as an anti-caking agent), maltodextrin, and citric acid as a stabilizer (or antioxidant, in effect). The National Agriculture Development Company (NADEC), one of the largest dairy companies in the Kingdom, was approached for this study, and its Board of Trustees agreed to take part by fortifying UHT-treated (define UHT) milk with 30 mg ferrous bis-glycine chelate per liter, which supplies 6 mg elemental iron per liter.

Study sample

The Ministry of Social Welfare accommodates socially deprived children in several full-board dormitories in Riyadh City. Three meals are served daily for these children under the supervision of social workers. Milk is served with the meals daily. A well-equipped clinic is available in each of these dormitories. This setting makes it ideal for carrying out a study under controlled conditions. The approval of the Ministry and the consent of the guardians of these children were obtained to carry out this trial in two dormitories, one for girls and the other for boys, with a total population of 173 children aged 6–14 years. However only 131 children (79 males and 52 females with a mean age of 10.2 ± 1.7 and 9.5 ± 1.8 respectively) had complete data. Three girls were menstruating.

Acceptability and tolerance of the fortified milk

Acceptability of fortified milk was judged by tray assessment after meal consumption and records were kept on a daily basis. Gastric discomfort complaints were assessed by questionnaire every week. The questionnaire included questions about bloating, colic, and stool pigmentation.

Hemoglobin and ferritin estimation

A sample of 10 mL of venous blood was drawn from each child before the start and at the end of the three-month study period. Five mL of each blood sample were added to EDTA-treated tubes for hemoglobin determination and the other 5 mL were kept in plain tubes for one hour; the serum was separated and kept at –20°C until used for ferritin estimation. Hemoglobin was estimated electronically by Coulter-Hemoglobinometer Machine, model ZF (26) (Coulter Electronics Ltd.; Luton, Beds, England). Ferritin was estimated by radioimmunoassay using Amerlite Ferritin Kit (Ortho-Clinical Diagnostic; Amersham, UK).

Fortified milk administration

Milk is a regular food item offered to all children with the three main meals. Plain, chocolate, and strawberry are the typical flavor additives used in milk commonly available in vending machines in these dormitories; consequently these flavor additives were used to avoid monotony. The flavored fortified milk was packed in 200 mL UHT-treated containers. Each child, anemic or not, received one liter daily (6 mg Fe) for 90 days. The reason for providing the nonanemic children with fortified milk was to avoid logistic problems and to eliminate bias and discrimination among children. This schema also made it possible to check the response (Hb levels) between "before treatment" and "after treatment" of nonanemic subjects. Milk distribution and consumption was done under the supervision of the social workers in these dormitories.

Table I: Distribution and mean (± SD) of Hemoglobin and Median and Range for Ferritin of Male and Female Children Pre- and Post-Treated with Ferrous Bis-glycine Chelate

	Pre-treatment	Post-treatment	p Value
Hemoglobin (g/dL)			
Male			
< 12 yrs n (%)	20 (25.5)	4 (5.0)	
> 12 yrs n (%)	59 (74.5)	75 (95)	
Mean (± SD)	12.44 ± 0.879	12.90 ± 0.823	< 0.0001*
Female			
< 12 yrs n (%)	12 (23.0)	5 (9.6)	
> 12 yrs n (%)	40 (77.0)	47 (90.4)	
Mean (± SD)	12.48 ± 0.855	13.00 ± 0.752	< 0.0001*
Ferritin (μg/L)			
Male			
< 12 yrs n (%)	6 (8.8)	4 (5.9)	
Median	27.7 (6.5–101.5)	26.5 (7.1–140.3)	NS
Female			
< 12 yrs n (%)	7 (21.2)	4 (12.1)	
Median	24.5 (4.8–80.7)	28.0 (8.0–111.1)	< 0.004**

* Paired t test; ** Wilcoxon signed rank test;
NS = Not Significant.

Exclusion criteria

Children who did not consume the fortified milk more than 90% of the time during the specified study period, or those with genetically predisposed anemia as shown by their medical record, were excluded from final data analysis.

Statistical analysis

Statistical analysis was done using the program Statistica by Stat Soft. Basic statistical data, including frequency distribution and paired "t" analysis to test for Hb level difference before and after treatment, were used. Nonparametric Wilcoxon signed ranks was used to test for significant differences between the two ferritin levels.

Results

Acceptability and tolerance of the fortified milk

The fortified milk was well accepted by the children. The preference for the flavors was in the order of chocolate > strawberry > plain milk. No organoleptic changes were detected in the chocolate-flavored milk. Moreover, with the intake of a dose of 6 mg iron per liter of milk per day, no gastrointestinal side effects among the children were observed with this chelate, and no detectable change in the organoleptic properties of the fortified milk was noticed after three months of storage.

Hemoglobin and ferritin levels

Table I shows that 25.3% of the male and 23.0% of the female children had Hb levels below 12 g/dL at the beginning of the study, while these figures fell to 5.0% and 9.6%, respectively, after consumption of fortified milk for three months. There was a statistically significant incremental response in hemoglobin level after the consumption of the fortified milk by both male and female groups ($p < 0.0001$).

Figures 1 and 2 show the Hb frequency distribution (%) curves of male and female children after consumption of ferrous bis-glycine fortified milk. Posttreatment curves were shifted to the right relative to the pretreatment curve, indicating a positive response of Hb to the consumption of ferrous bis-glycine fortified milk for the children with Hb levels less below 12 g/dL. Figure 2 indicates that among the nonanemic girls (> 12 g/dL), fortified milk had little effect on the Hb level. However, for the nonanemic boys (Fig. 1), there was slight improvement in hemoglobin level. The rise in serum ferritin was statistically significant only for the females ($p < 0.004$). The results of

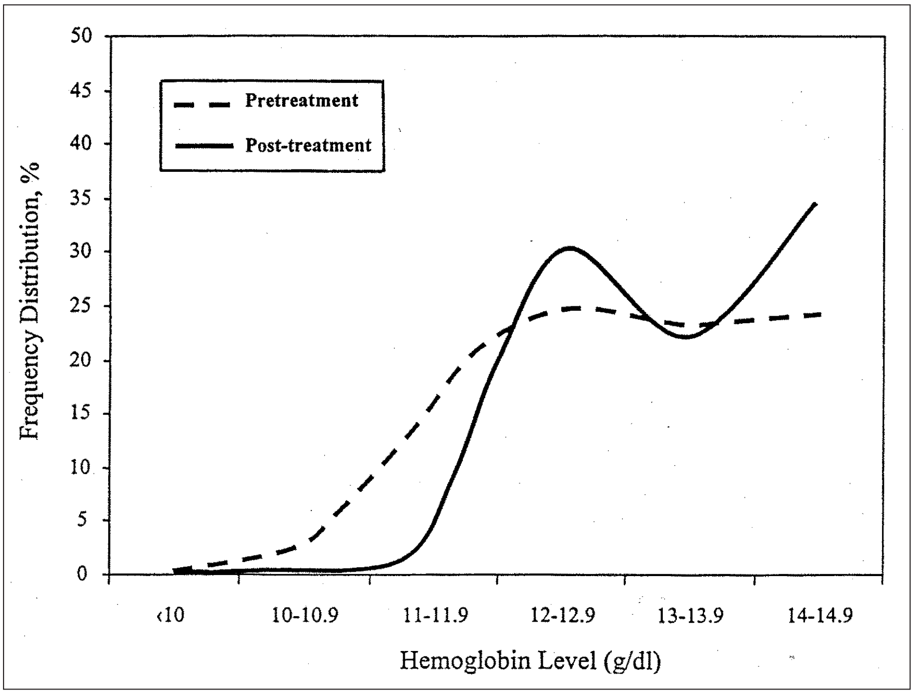

Figure 1: Effect of three months' consumption of milk fortified with ferrous-bis glycine chelate on hemoglobin level (Males).

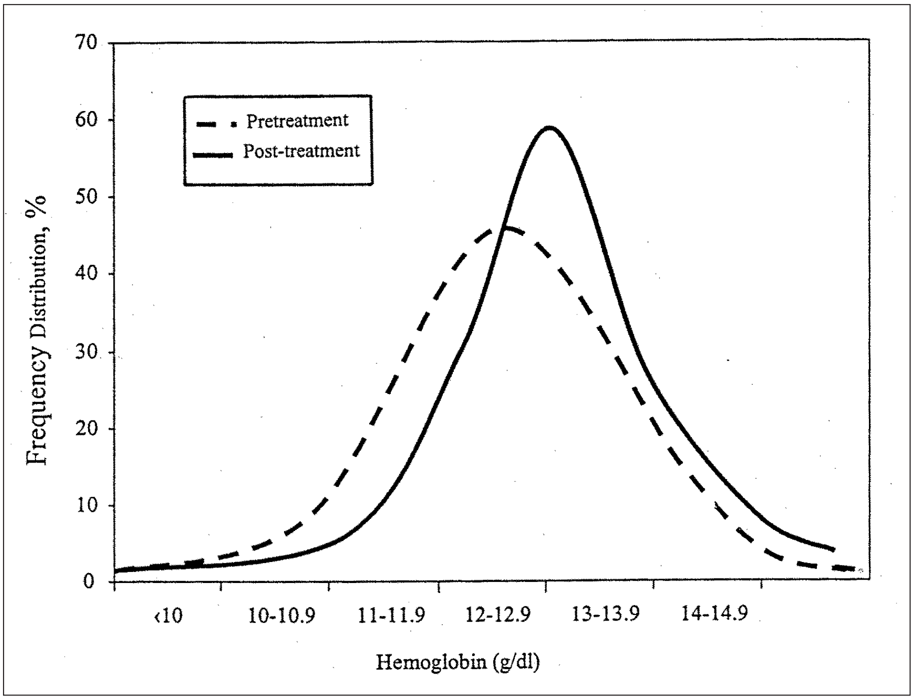

Figure 2: Effect of three months' consumption of milk fortified with ferrous-bis glycine chelate on hemoglobin level (Females).

ferritin estimation (Table I) showed that 8.8% of the male and 21.2% of the female groups presented with serum ferritin levels below 12 mg/L before treatment. These percentages fell to 5.9% and 12.1% post treatment respectively.

Figure 3 shows the frequency distribution (%) of serum ferritin. At levels of more than 12 µg/L, no difference in ferritin serum concentrations in male or female groups, as compared with post-treatment values, was noticed. In the pretreatment group no females showed a level between 60 and 80 µg/L. However 15.2% of them showed a value between 60 and 80 µg/L in the post-treatment period. No values above 140.3 µg/L were detected (normal range for females = 12–150 µg/L).

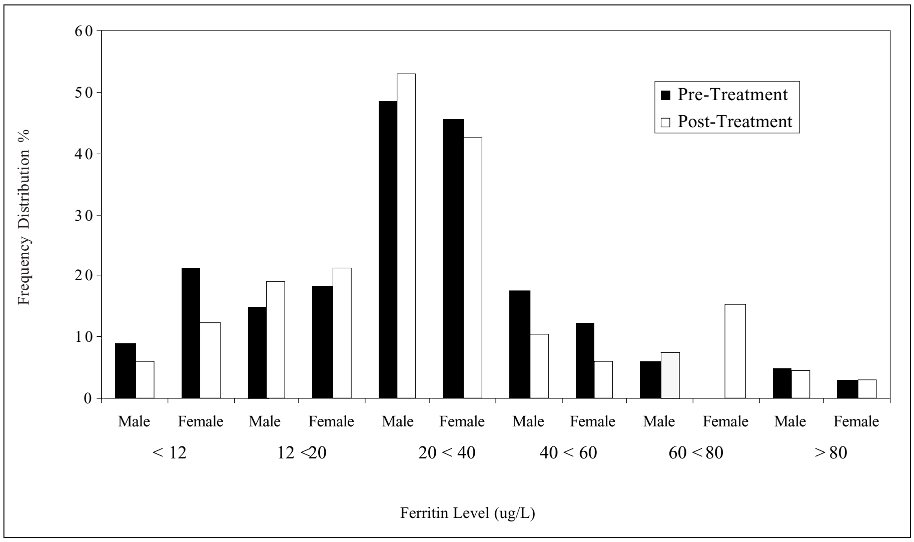

Figure 3: Frequency distribution (%) of ferritin levels among male and female children pre- and post-treated with ferrous bis-glycine chelate.

Discussion

Iron deficiency has far-reaching consequences on systemic functions, apart from the well-known feature of anemia. The nonhematologic manifestations of iron deficiency were reported to be associated with alterations in cellular function, growth, motor development, behavior and cognitive function in children [12]. The level of immunocompetence against infection is compromised and there are consequences for physical work and metabolic stress. Altered fetal outcome and premature labor in pregnant women was also reported [27]. Food fortification has been shown to be an effective strategy for treatment and control of IDA [28]. The result of this study indicates that the ferrous bis-glycine chelate-fortified milk supplying 6 mg elemental iron per day for three months has definitely improved the hemoglobin level in both male and female anemic children. This is similar to Pineda's [25] findings, which showed the effectiveness of iron bis-glycine chelate in the treatment of iron deficiency anemia. However, for the serum ferritin concentration, there was statistically significant improvement in the girls but not in boys. This difference between the two groups is suggestive of low pretreatment iron stores in the females.

No off flavors were detected, indicating that this chelate did not alter the organoleptic properties of milk. Previous reports [29–31] showed that bis-glycine chelate is a fortificant that can be added to the fluid phase of high-fat content foods such as milk or milk products without inducing prompt peroxidation or rancidity.

It has been shown that ferrous sulfate, when added to milk, has low bioavailability; this was attributed to the presence of two inhibitory factors, namely calcium [32] and milk protein casein [33]. In a series of trials in Chile in which ferrous sulfate-fortified infant formula was investigated, the improvement of iron status was only modest in the absence of vitamin C. However it improved considerably when vitamin C was added to the formula [34]. Adding vitamin C to fluid milk has been reported to degrade rapidly to diketogluconic acid, leading to changes in flavor and many soluble iron compounds that have been shown to rapidly produce off flavors. This was attributed to the promotion of lipolytic rancidity, oxidative rancidity by the oxidation of free fatty acids, and partial or complete loss of vitamins A, C, and beta carotene [35, 36]. The result of this study showed clearly that the mean Hb level of the anemic children had improved within the three-month study period, even in the absence of iron absorption enhancers at the level of 6 mg Fe per day. Although we did not investigate the bioavailability of ferrous bis-glycine chelate in relation to other iron fortificants, this result is indicative of good bioavailability of iron from this iron chelate, leading to improvement in iron status of anemic children.

Approximately 3.3 million women of childbearing age and 240 000 children aged 1–2 years have IDA in the US [37]; conversely up to one million persons in the US may be affected by iron overload due to hemochromatosis [38, 39], a genetic condition characterized by excessive iron absorption, excess tissue iron stores, and potential tissue injury. Olivares *et al* [19] showed a significant correlation between (log) iron absorption of ferrous bis-glycine with (log) serum ferritin ($r = -0.60$, $p < 0.03$) suggesting that iron bis-glycine chelate bioavailability is indeed affected by iron stores. Bovell-Benjamin [23] reported a similar correlation ($r = -0.61$, $p < 0.03$) indicating an effective downregulated iron absorption from iron bis-glycine, caused by (?) the iron reserve of iron-sufficient individuals. The result of this study (Fig. 3) shows that there is no difference between pre- and post-treatment serum ferritin levels at concentrations of > 80 μg/L among these children. Although no determination of iron absorption from this chelate has been carried out yet, this result suggests that downregulated iron absorption via the iron reserve of iron-sufficient individuals needs further verification.

One of the drawbacks of this study is that no anemic control group receiving placebo or other fortificants (i.e. $FeSO_4$) was investigated. The decision to omit control groups was made for statistical reasons as the number of anemic children in this study was small.

In conclusion, it may be stated that milk is an appropriate vehicle to be fortified with ferrous bis-glycine chelate without alteration of its organoleptic properties, and without gastrointestinal discomforts or side effects. In this study, the Hb status of anemic children was improved without the use of iron absorption enhancers.

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